

III. Bovine Corneal Opacity and Permeability Test Method

Comments provided by the Members of the COLIPA/SCAAT Eye Irritation Task Force:

Christine Van Den Berghe (L'Oreal, Aulnay Sous Bois, France)

Freddy Van Goethem (Johnson & Johnson Pharmaceutical Research and Development, Beerse, Belgium)

Technical comments

General comments

1. BCOP Test Method Rationale

Page 57 § 1.1.2.: it is mentioned that some materials can cause serious corneal injury without appearing to change opacity or permeability immediately (e.g. mustard gas). Is this a specific observation for mustard gas or are other examples (agents) known which can induce damage without changes in opacity and/or permeability? Injury (cell death) without any change in opacity and/or permeability is very rare.

Page 58 paragraph 1.1.3: the rabbit and bovine cornea both differ from the human cornea, however, the use of the in vivo rabbit test has apparently protected human populations from serious injury for many years. We agree with this paragraph but future validation studies need to be specify/detail their main objective:

- to predict human response **or** rabbit response ?
- to protect people **or** to predict the eye irritating potential of compounds?

Page 59 § 1.2. and 65 § 4.5: although the BRD points out clearly that there are no data comparing the results in the in vivo rabbit test to similar human exposure, (except for very mild substances). We agree with the expert panel that ICCVAM should make an effort to obtain and consider information on human topical ocular chemical injury. We are of the opinion that it is worthwhile to explore the possibility to collect relevant data from accidental human exposure by examining the injury databases maintained by the Poison Control Centers and the Department of Labor. The United States Eye Injury Registry (USEIR) may be another source of such information.

2. BCOP Test Method Protocol Components

Page 59 § 2.1.1: the panel report describes that the optimum age range for cattle should be determined; however, until this is evaluated, eyes should be obtained from young adult animals of 18-48 months of age. An additional possibility is to use corneas from animals less than 12 months old which can result in different advantages: (i) in Europe there is no concern about Bovine Spongiform Encephalopathy (BSE) in animals < 12months (safer & less complex administration) and (ii) the quality of the corneal tissue is higher (decreased variability).

Page 59 § 2.1.1: eyes can probably be stored up to 12 h (refrigerator), but this needs to be confirmed by careful examination of the eyes prior to testing. In this context, it can be mentioned that GAUTHERON developed a preservative medium which allows to store corneas up to 24h. Preservation procedure was described in *Gautheron et al. (1994). Interlaboratory assessment of the bovine corneal opacity and permeability (BCOP) assay. Toxicology In vitro 8, 3, 381-392*. This can be considered a better approach compared to storage in a refrigerator.

Page 60 § 2.1.1: the use of an improved cornea holder (*Ubels et al. 2002, 2004*) may indeed provide the appropriate dimensions to maintain the natural curvature of the cornea. Indeed, trying to clamp small size corneas in standard holders, can result in overstretched (wrinkled) tissue and variable results. However, we think that the correct use of standard holders also can provide adequate test results (e.g. holders with a reduced diameter should be used when corneas from young animals are mounted).

Page 61 § 2.1.4: the duration of exposure needs to be standardized (10 minutes - 4 hours) for certain types of test materials. The expert panel correctly mention that the protocol does not recommend a 3-minute exposure for volatile solvents. In addition, other exposure times used within (cosmetic) industry (30, 60 and 120 minutes) can be described since they are able to improve accuracy and prediction ability (*Harbell and Curren, 1998*). Exposure times can vary depending on the purpose of the study or material type (see WORKSHOP SUMMARY *Curren and Harbell 1998, In vitro and Molecular Toxicology 11 (4), 315- 351*).

Page 63 § 2.7: the osmolarity and pH of test solutions should indeed be measured and recorded. However, determination of pH and osmolarity is not always possible (i.e. when test formulation is a cream, suspension or neat solid). Solutions with osmolarity above 1000 are

known to damage corneal epithelium => it would be useful to include the corresponding reference.

Page 63 § 2.7 and 73§ 12.2: the implementation of histopathology as extra endpoint can indeed be an added value. However, when screening numerous test compounds and formulations, this approach should only be considered case by case. For example, in order to avoid false negative results when there is neither opacity nor alteration of permeability. If there is an obvious increase in opacity and/or permeability, histological analysis is not relevant and will not give complementary information.

3. Substances Used For Previous Validation Studies of the BCOP Test Method

=> no remarks

4. In Vivo Reference Data Used for an Assessment of Test Method Accuracy

=> no remarks

5. BCOP Test Method Data and Results

Page 67 § 5.5: The expert panel's data evaluation indicates that alcohols and ketones are the problematic substances, but additional physicochemical data are needed to refine this evaluation. Indeed, one can question if all alcohols and all ketones are problematic?

6. BCOP Test Method Accuracy

Page 68 § 6.2: we agree that exploration of the effect of physicochemical properties is limited. In particular, the lack of prediction of BCOP for ketones, alcohols and solids need to be investigated and it could be explained first by the lack of prediction of the Draize test to human eye irritation.

7. BCOP Test Method Reliability (Repeatability/Reproducibility)

Page 69 § 7.2: CV values should indeed be used with care. Comparing the means of the CVs of a set of results with predominantly high scores with a set of results with predominantly low

scores is inappropriate. Significant differences between laboratories can be evaluated by the use of ANOVA techniques.

8. Test Method Data Quality

=> no remarks

9. Other Scientific Reports and Reviews

=> no remarks

10. Animal Welfare Considerations (Refinement, Reduction, and Replacement)

=> no remarks

11. Practical Considerations

Page 72 § 11.3: the expert panel want to reflect public comments submitted by S.C. Johnson & Son, Inc. in December 2004 on the costs and time comparisons with the Draize test. Indeed, it is difficult to compare cost and time between in vitro and in vivo test models; this comparison might induce confusion. The use of alternatives is first of all an ethical and scientific choice, not an economical. In vitro approach is totally different from in vivo study.

12. Proposed Test Method Recommendations

General comment: in order to improve the accuracy and reliability (repeatability/reproducibility) of the proposed BCOP protocol, several changes are recommended including use of the larger holder similar to that suggested by Ubels et al. (2002), re-examining the use of the calculated total score when the endpoint is serious injury only, changes to the medium used to bathe the eyes, avoiding use of antibiotics, and appropriate ages of donor animals. However, we agree with the expert panel that there should be more discussion of the variability of the rabbit data. Because of the known variability in the rabbit test, it is not possible from the data presented to determine if the

inconsistencies between the two tests are due to "failure" of the in vitro test method or a misclassification by the single in vivo result provided. Thus, before the proposed protocol is changed or new optimization/ validation studies are set-up, the accuracy of the in vivo test should be further evaluated. In addition, proposed protocol modifications should be checked with experts for rationale and/or additional data [e.g. Ubels holders: is there scientific proof (data) that better data are generated compared to standard holders??].

13. BCOP BRD References

=> no remarks

14. Panel Report References

=> no remarks
